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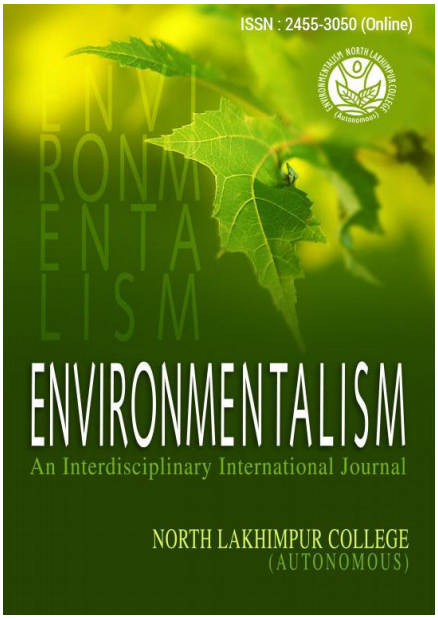

A study on total flavonoids, phenol contents and phytochemical screening of leaves extract of *Desmodium caudatum* (Thunb.) DC, *Streblus aspera* Lour. and stem of *Plumeria acuminata* Ait.

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A STUDY ON TOTAL FLAVONOIDS, PHENOL CONTENTS AND PHYTOCHEMICAL SCREENING OF LEAVES EXTRACT OF *DESMODIUM CAUDATUM* (THUNB.) DC, *STREBLUS ASPERA* LOUR. AND STEM OF *PLUMERIA ACUMINATA* AIT.

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Abstract

The present study is an attempt to investigate the phytochemical constituents and estimation of total flavonoid and phenol contents of three different medicinal plant *Desmodium caudatum*, *Streblus aspera* and *Plumeria acuminata* that are used by the Mising tribes of Assam, India for curing reproductive related disorders. For phytochemical screening, some common and available standard tests were done. Aluminum chloride colorimetric method was used for total flavonoid determination using Quercetin as standard. Determination of total phenol was done by Folin-reagent method using Gallic acid as standard. Phytochemical screening showed the presence of active compounds in high concentration, such as alkaloids, flavonoids, phenolic compounds, saponin in addition to carbohydrate and protein. Among the three plant investigated, the total flavonoid content was found to be highest in *S. aspera* (971±12.41) µg/g quercetin equivalent of methanol extract in colorimetric method, than the other two; while the total phenol content was found to be highest in *D. caudatum* (97.00±3.46) µg/g gallic acid equivalent of methanol extract in Folin-reagent method. All these results show positive aspect of these plants to be used as medicine as these plant compounds are known to have different pharmacological activities.

Keywords: Phytochemical screening, Folin-reagent, Quercetin, Gallic acid, flavonoid, phenol.

1 Introduction:

Plants are used as medicine in our country since time immemorial. From the ancient period different parts of plants

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have been used for curing different types of ailments caused by microorganisms. In our country, the traditional system of medicine plays an important role in health care of rural people for all types of ailments. The healing power of traditional herbal medicines have been realized and documented since Rig veda and Atharba veda (Bhattacharjya and Borah 2008). According to an estimate, more than 120 or so plant based drugs prescribed for use throughout the world come from just 95 plant species (Lewington 1993). This group approximately consists of 30,000 species belonging to 5000 genera and more than 1000 families and sub-families. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. Some of the most important of these bioactive constituents of plants that are capable of producing physiological changes in the body are alkaloids, tannins, flavonoids, and phenolic compounds (Hill 1952). These compounds are synthesized by primary or to a certain extent the secondary metabolism of living organisms. Secondary metabolites are chemically extremely diverse compounds with doubtful function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas. There is a wide range of plant parts possessing a variety of medicinal property, such as flowers, fruits, leaves,, stems, barks and roots extracts (Nair *et al.* 1976). About 25% of prescribed drugs available today in the world are of plant origin. They are of great importance to the health of individuals and communities. Many of these plants are used traditionally by the local tribes of this region since ancient time. They also use these plants for performing many of their rituals and in festivals as food and as spices. They are also sometimes added to foods which are meant for pregnant and nursing mothers to cure some of the reproductive problems (Okwu 2001). To know about the biological activities of these traditional medicinal plants, phyotochemical screening is outmost essential. Nowadays, secondary plant metabolites have been extensively investigated as a source of medicinal agents (Krishnaiah *et al*. 2009). In spite of the recent trends in modern health care system, still approximately 80% people depend on traditional plant based medicines for their initial health care needs in developing countries. In order to encourage the use of herbal medicines and the determination of their potentials, the studies of medicinal plants should be more intensified particularly those used as folk medicines (Nair *et al.* 2009). Therefore in this present study, an attempt has been made to investigate the fundamental scientific bases for the use of three medicinal plants used by the Mising tribe of Assam (India) by defining and finding out the phytochemical constituents present in these plants.

2 Materials and methods

2.1 Collection and identification of plant

For this present investigation the plant materials i.e., the leaves of *Streblus aspera*, the leaves of *Desmodium caudatium* (Thunb) DC and stem of *Plumeria acuminata* Ait. was collected from Dhemaji and North Lakhimpur district, Assam, India in the month of January, 2014. Standard method regarding collection and preservation of plant specimens was followed (Jain and Rao 1977). Voucher specimens of plants were collected, organized and herbarium sheets prepared. It was then identified and authenticated by the Department of Botany, Gauhati University, Assam, India. A voucher specimen of each plant sample was deposited at the department of Botany, Guwahati University for future reference.

2.2 Description of the plant under investigation

2.2.1 Streblus aspera Lour- Also known as Saura, Thoura, Phuhura, Pan saura, toothbrush tree belongs to the family Moraceae. It is a well known ethnomedicinal plant and is used in Ayurveda and other folk medicines. They are used for the treatment of different types of ailments such as leprosy, filariasis, toothache, diarrhoea and cancer (Sahu *et al.* 2010; Reang *et al*. 2016). It is distributed in tropical and subtropical regions of World and is also found all over Assam. (Acc. No.-GUBH-17878/10-03-2015).

2.2.2 Desmodium caudatium (Thunb.) DC – Belonging to the Papilionaceae (Fabiaceae) family, this plant is an annual under-shrub grown mostly on shady damp places. It is found mostly all over Assam. Commonly known as bhuter chira, hoary trick trefoil-leaf, biyoni sapota. The genus Desmodium contains about 350 plant species which are used for both feeding stuffs and herbal medicines. Among them about 30 species have been phytochemically or pharmacologically investigated (Ma *et al.* 2011). (Acc. No.--GUBH-17877/10-03-2015).

2.2.3 Plumeria acuminata Ait. –Commonly known as Boga gulonchi, Boga gulochi, Sweta gulanchi, White frangipani. It is a small soft wooded deciduous tree or shrub, native to many parts of America and Asia belonging to Apocynaceae family. The fragrant flower of the plant is white in colour and often planted as ornamental near temples and garden. The fruits are also edible. Found mostly in different parts of Assam. The milky juice of the plant is employed for the treatment of inflammation. The plant material (leaves, bark, flower and oil) is also extensively used as a remedy for pain, fever, diarrhea, purgative and as a cure for itch in many countries (Hua and Geng-Tao 1992; Formica and Regelson 1995; Aruoma and Cuppett 1997). (Acc. No.- -GUBH-17872/10-03-2015).

2.3 Preparation of the plant material

The collected plant parts i.e., the leaves of *Streblus aspera* and *Desmodium caudatum* were washed properly and separated from undesirable materials. The stem of *Plumeria acuminata* was taken and removed of its bark. Then the stem was sliced into small pieces. The samples were air-dried (shade) to constant weight at room temperature (30°C) for a week after which it was grinded to a uniform powder with the help of a suitable grinder. The powder plant material was then stored in an airtight container and kept in a cool and dry place for future analysis.

2.4 Extraction of plant material

The methanol extracts were prepared by soaking 100 g each of the dry powdered plant materials in 200ml of methanol in a Soxhlet extractor by continuous hot percolation for about 36 hour. The whole mixture then was filtered through Whatman No. 1filter paper. The filtrate were concentrated and evaporated to dryness using a rotary evaporator with the water bath set at 40°C. It rendered a gummy (paste) concentrate of dark brown colour for *Plumeria acuminata* and dark green colour for *Streblus aspera* and *Desmodium caudatum.* The gummy concentrate was designated as crude extract or methanolic extract which were then weighed.

Percent of yield (Harborne 1998) was calculated in terms of initial dried plant material as follows:

Extract yield % = $(W_1/W_2) \times 100$

(Where, W_1 is net weight of powder in grams after extraction and W_2 is total weight of plant powder in grams taken for extraction).

2.4 Qualitative phytochemical analysis

The methanolic extract of the plants were analyzed to identify the presence of various phytoconstituents by using standard procedures (Kokate 1986; Evans 1989; Harborne 1998). The crude extracts were qualitatively tested for the presence of chemical constituents using different reagents and chemicals which are of analytical reagent grade.

2.4.1 Test for detection of Carbohydrate

Molisch's test: To 2ml of the extract, 2 drops of alcoholic α-naphthol was added. After that 2ml of concentrated sulphuric acid was poured along the side of the test tube. A purple or violet ring appears at the junction of the two liquid confirms the presence of carbohydrate.

Benedict's test: To 2ml of the plant extract 5 ml of Benedict's solution was added in a test tube. The mixture was boiled for 2 min and allowed to cool spontaneously. Formation of red precipitate indicates the presence of sugar.

Fehling's test: To 2 ml of the extract, equal volume of Fehling A and B solution was added and the solution was boiled for 2 minutes. Formation of brick red precipitate indicates the presence of sugar.

Iodine test: 2 ml of the extract was added to 2 drops of Iodine solution. Appearance of dark or purple colouration indicates the presence of starch.

2.4.2 Test for detection of Alkaloids (the extract was dissolved in dilute Hydrochloric acid and then filtered)

Mayer's test: To 2 ml of the filtrate, 2ml of Mayer's reagent was added. Formation of yellow/ whitish/ cream colour precipitate indicates the presence of alkaloids.

Hager's test: 2 ml of the filtrate was treated with Hager's reagent. Formation of yellow colour precipitate confirms the presence of alkaloids.

Wagner's test: 2ml of the filtrate was treated with Wagner's reagent. Appearance of red precipitate indicates the presence of alkaloids.

2.4.3 Test for detection of Glycosides

Killer-Kilani test: The extract was mixed with 2/3 ml of glacial acetic acid and added 1-2 drops of 2% $FeCl_3$. Presence of a brown ring at the interface establishes the presence of glycosides.

Salkowski's test: 2ml of chloroform was added to the extract and then 2-3 drops of concentrated sulphuric acid was added and shaken gently. Development of reddish brown colour indicates the presence of glycoside.

Libermann's test: To 2ml of the extract, 2ml of acetic acid and 2ml of chloroform was added. The mixture was then cooled in ice. Concentrated sulphuric acid was poured into the mixture carefully. A colour of the mixture change from violet to blue to green indicates the presence of steroidal nucleus, i.e., glycone portion of glycoside.

2.4.4 Test for detection of proteins and amino acids

Xanthoproteic test: A few drops of concentrated nitric acid was added to 2ml of the plant extract. Yellow colour formation of the solution indicates the presence of proteins.

Millon's test: 2ml of Millon's reagent was mixed with 2ml of the extract. Appearance of white precipitate which turns red upon gentle heating, confirms the presence of protein.

Ninhydrin test: The extract was boiled for a few minutes with 2ml of 0.25%w/v ninhydrin reagent. Formation of blue/violet colour suggests the presence of amino acids and protein.

2.4.5 Test for detection of Phenol

Ferric chloride test: The extract was treated with 2-3 drops of ferric chloride solution. Appearance of bluish black colour indicates the presence of phenol.

To the extract, strong potassium dichromate solution was added. Development of a yellow colour precipitate indicates the presence of phenolic compound.

2.4.6 Test for detection of Tannin

To a small quantity of the extract lead acetate solution was added. Development of a white precipitate indicates the presence of tannin.

To a small quantity of the extract, potassium ferric cyanide and ammonia solution was added. Appearance of a deep red colour indicates the presence of tannins.

2.4.7 Test for detection of Flavonoids

Alkaline reagent test: The extracts were treated with 3-4 drops of 2% sodium hydroxide solution. An intense yellow colour develops which turn colourless on addition of few drops of dilute acid, proves the presence of flavonoids.

Shinoda test: The plant extract was mixed with a few fragments of magnesium ribbon. To the mixture concentrated hydrochloric acid was added drop wise. Appearance of pink scarlet colour after a few minutes indicates the presence of flavonoids.

Lead acetate test: A few drop of lead acetate solution was added to 2ml of the plant extract. Appearance of yellow colour precipitate indicates the presence of flavonoids.

2.4.8 Test for Saponin

Froth test: The extract was diluted with 5 ml of distilled water and was shaken vigorously. Formation of stable foam indicated the presence of saponin.

2.4.9 Test for detection of terpenoids

The plant extract was dissolve in2-3ml of chloroform and was evaporated to dryness. 2ml of concentrated sulphuric acid was added to this and was heated for about 2 minutes. Development of a greyish colour suggests the presence of terpenoids.

2.5 Quantitative determination of phenol by Folin-reagent method

Folin-Ciocalteu reagent method was followed for determination of total phenol content of these plant extract (Hossain *et al.* 2011). 10% Folin-Ciocalteu reagent was prepared by adding 10ml of Folin-Ciocalteu reagent in 90 ml distilled water. 1 mg each of the plant extract was dissolved in 1 ml of methanol. In order to make 7.5% Na_2CO_3, 3.75gm of Na_2CO_3 was dissolved in 50 ml distilled water. Then 500µl of plant extract each was taken in a test tube

and 2.5ml of Folin-Ciocalteu reagent (10%) was added to it. Then the test tube was incubated at room temperature in the dark for about 5 minutes. Then finally 7.5% Na_2CO_3 (2ml) was added to the solution and mixed thoroughly. The test tubes were again allowed to stand undisturbed for about 1 hour at room temperature. Then the absorbance was measured for all the solutions at a constant wavelength of 765nm in a UV-spectrophotometer. Total phenol content was then determined from the Gallic acid standard curve and the equation thus obtained and were expressed in terms of Gallic acid equivalent (µg/g of extracted compounds). Measurement of the samples was made in triplicate.

2.6 Preparation of Gallic acid standard curve

For preparation of the Gallic acid calibration curve, Gallic acid 10mg was dissolved in 10 ml methanol. This was the concentration of 10mg/10ml (1g/l). Then by adding methanol, serial concentrations were prepared (0.01, 0.02, 0.03, 0.04, 0.05 mg/ml). Then the same procedure as described above was also followed for gallic acid standard. Then the absorbance was measured for all the standard solution at the same constant wavelength of 765nm in a UV-spectrophotometer.

2.7 Quantitative determination of Flavonoids by Aluminiun Chloride Colorimetric method

Aluminium chloride colorimetric method with slight modification was followed for determination of total flavonoids contents of crude extracts of different plants as described by Hossain *et al.* (2011). Each plant extract 5ml was dissolved in 5mlof methanol. 5% sodium nitrate was prepared by adding 0.5gm sodium nitrate in 10ml distilled water. 10% aluminium chloride was prepared by adding 1gm aluminium chloride in 10ml distilled water. Then 1M sodium hydroxide was prepared by adding 0.2g sodium hydroxide in a volumetric flask and volume made up to 50ml with distilled water. The extract of different plants 0.5ml each was taken in test tube and added 2ml water and 0.15ml sodium nitrate (5%). All the test tube was allowed to stand for about 5 minutes. Then 0.15ml of 10% aluminium chloride was added to the test tube and waited for 6 minutes for the reaction to occur. 1ml sodium hydroxide was added and finally volume of the tube was made up to 5ml by adding 1.2ml of water. The test tube was incubated for about 30 minutes and the absorbance measured at a wavelength 510nm for all samples with the help of a spectrophotometer. All samples for estimation of total flavonoid content was carried out in triplicate and results averaged. Quercetin was used for determination of the standard calibration curve. A calibration curve was developed by measuring the absorbance of the dilutions at 510 nm (λ max of quercetin) with a Systronic UV-VIS Digital spectrophotometer, Type 118. Total flavonoid content was then determined from the standard curve and the equation thus obtained, and were expressed as quercetin equivalent (µg/g of the extract).

3 Results

Using standard protocol the phytochemical analysis of the plant was done. Preliminary investigations on the phytochemical constituent showed the presence of various types of plant metabolites in the plant extract.

3.1 Extractive yield

Different solvents give dissimilar yield depending upon their solubility in a particular solvent and the phytoconstituents present in them. Therefore, choice of a particular solvent for extraction is very important as also reported by Kaneria and Chanda (2012). Efficiency of extraction is a very important step towards the discovery of

bioactive components from plant material. In the present study, dry powder of the different plant material was extracted with methanol as the solvent. Percent yield of the plant extract was then calculated (Harborne 1998):

The percentage yield of extracts ranged from 6.66%w/w in *Streblus aspera*, 7.34%w/w in *Plumeria acuminata* and 9.02%w/w in *Desmodium caudatum.*

3.2 Phytochemical screening

The crude extract was subjected for various chemical group tests and identified to have different types of important chemical constituents.

Table-1 Qualitative phytochemical analysis of *P. acuminata, D. caudatum and S. aspera* (Present=++; Trace=+; Absent=--)

Sl. No.	Secondary metabolite	Name of the Test	Result		
			Plumeria acuminata	*Desmodium caudatum*	*Streblus aspera*
1.	Alkaloids	Mayer's test	++	+	++
		Wagner's test	+	++	++
		Hager's test	+	++	++
2.	Saponin	Froth test	++	+	+
3.	Phenol	Ferric chloride test	+	+	++
4.	Glycosides	Keller-Kilani test	--	+	+
		Salkowski's test	--	+	+
		Liebermann's test	--	+	+
5.	Phytosterols	Salkowski's test	--		
		Liebermann-Burchard test	--		
6.	Terpenoids	Chloroform+Conc.H_2SO_4	+	--	
7.	Tannin	Lead acetate test	--	+	--
8.	Flavonoids	Alkaline reagent test	++	++	++
		Shinoda test	++	+	+
		Lead acetate test	+	+	+
9.	Carbohydrate	Molisch's test	++	++	++
	Reducing sugar	Benedict's test	++	++	++
		Fehling's test	++	++	++

10.	Proteins and amino acids	Xanthoproteic test	++	+	++
		Millon's test	++	+	+
		Ninhydrin test	++	+	+

The result for phytochemical screening of methanol extracts from the leaves of *D. caudatum*, *S aspera* and stem of *P acuminata* are shown in Table 1 above. Results have showed the presence of flavonoids, alkaloid, saponins, and phenol in addition to carbohydrate and proteins in all the three types of plant extract. Cardiac glycosides were found to be present in *D. caudatum*, *S aspera* but absent in *P. acuminata* extract. Moreover tannin was found to be present in *D. caudatum* only while it was absent in *P. acuminate* and *S aspera.*

3.2 Quantitative estimation of total Phenol content by Folin Reagent Method

The total phenol contents of the three crude plant extracts determined by Folin-Ciocalteu method were reported as gallic acid equivalents (Figure 1). From among the three crude extracts, methanolic leaf extract of *D. caudatum* contained the highest (97.00±3.46 µg/g) amount of phenol compounds followed by stem extract of *P. acuminata* (24.15±2.00 µg/g). The least amount of phenol content was found in the leaf extract of *S. aspera* (20.11±4.21 µg/g).

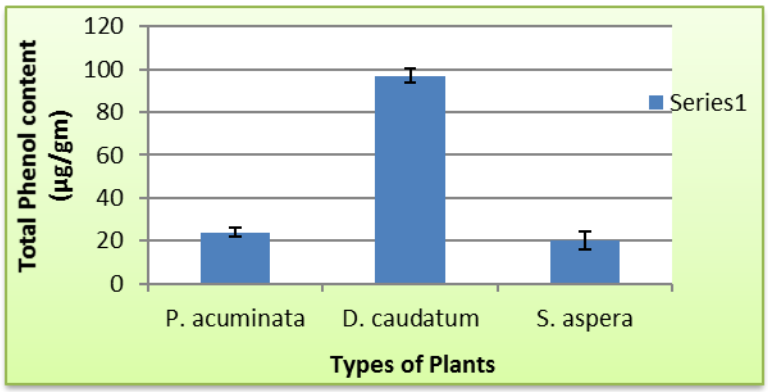

Figure 1 The total phenol contents in the three different plant extracts

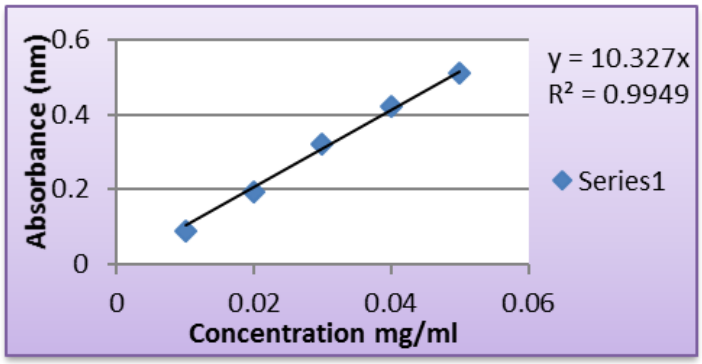

Figure 2 Gallic acid standard curve for determination of total phenol content in the plant extract

3.3. Total flavonoid contents by Aluminiun Chloride Colorimetric method

The result of total flavonoid contents of the three crude extracts of the plants is given in Figure 3. The total flavonoid contents in the three different crude extracts varied from 201 to 971 µg quercetin/g weight. Among the three crude extracts, methanol extract of *S. aspera* contained the highest (971±12.41 µg/g) amount of flavonoids content compounds followed by *D. caudatum* (510±14.26 µg/g). The least amount of flavonoid content was found in *P. acuminata* stem extract (201±8.41 µg/g).

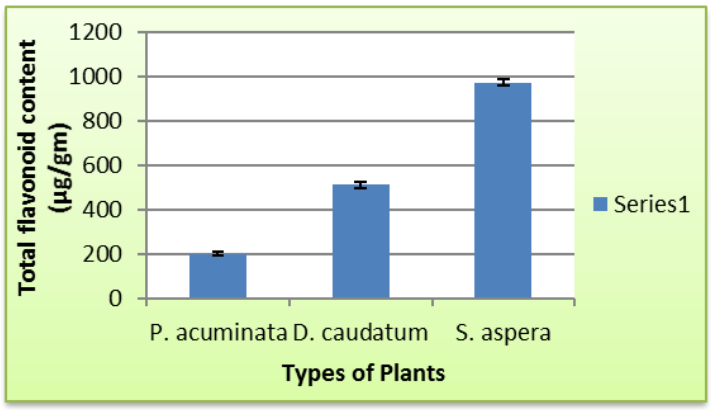

Fig-3: The total flavonoid contents in the three different plant extracts.

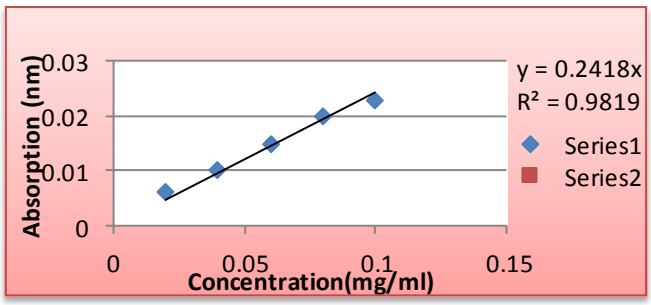

Fig-4: Calibration curve of quercetin standard for determination of total flavonoid content in the plant extract.

4 Discussion

Chemical constituents present in the plants or crude extracts are known to be biologically active ingredients. These chemical constituents or secondary metabolites components are directly responsible for causing different physiological activity such as anticancer, antimicrobial, antifungal and antioxidant (Harborne 1998; Hossain and Nagooru 2011). The present study on qualitative phytochemical analysis on the methanolic stem extract of *Plumeria acuminata* Ait. which was done using the standard protocol available in literature reveals the presence of saponin, flavonoids, phenolic compounds, and alkaloids in addition to carbohydrate and proteins. This study resembles the study undertaken by Gupta *et al.* (2006). The phytochemical screening on the leaf extract of the plant

Desmodium caudatum has also shown the presence of these compounds. In addition it also showed the presence of cardiac glycosides and tannin. Moreover, the evaluation of the phytochemical constituents on the methanolic leaf extract of *Streblus aspera* also revealed the presence of the above mentioned compounds such as saponin, flavonoids, phenolic compounds, cardiac glycosides and alkaloids, but tannin was found to be absent. Flavonoids are bioactive polyphenols with low molecular weight that are present in a variety of foods. The different types of flavonoids exerting a varied range of biological activities includes anti-allergic, anti-viral, cytotoxic anti-tumour, ailing neurodegenerative diseases and vasodilator action (Williams *et al.* 2006; Chebil *et al.* 2006; Tsuchiya 2010), anti-bacterial, anti-inflammatory (Cook and Samman 1996). They are also found to have a varied effect on fertility both in male and female (Ghosh *et al.* 2011). Phenols, also called phenolics, consist of a group of chemical compounds of a hydroxyl group (—OH) bonded directly to an aromatic hydrocarbon group the simplest of which include phenol/carbolic acid (C_6H_5OH). These are mainly distributed among the plant kingdom and are the most abundant secondary metabolites of plants. In recent times, plant phenolic acids have been considered to be a powerful antioxidants *in vitro* and found to be more effective antioxidants than Vitamin E, C and carotenoids (Rice-Evans *et al.* 1995; Rice-Evans *at al.* 1996).). The inverse relationship between the possibility of oxidative stress related ailments such as cardiovascular diseases; osteoporosis or cancer and vegetable and fruit intake has been moderately ascribed to phenolics (Hollman and Katan 1999; Scalbert *et al.* 2005). This compound is also known to have an effect on the fertility in animals. A number of workers have reported about the anti-fertility effect of certain plants having phenolic acids as their secondary metabolite. Some such plants includes, *Rivea hypocrateriformis* (Shivalingappa *et al.* 2002), *Butea monosperma* (Sindhia and Bairwa 2010) etc. Alkaloids are naturally occurring chemical compounds produced by a diversity of living organisms including bacteria, fungi, plants and animals. Well-known alkaloids including strychnine, quinine, , morphine, nicotine and ephedrine are found to have an extensive range of pharmacological activities like analgesic (e.g. morphine) (Sinatra *et al.* 2010), anticancer (e.g.homoharringtonine) (Kittakoop *et al.* 2014), antimalarial (e.g. quinine), antibacterial (e.g.chelerythrine) (Cushnie *et al.* 2014) etc. In the field of reproduction many alkylating agents also are found to produce infertility both in animals and man (Miller 1971; Fairley *et al*. 1972).

Saponins, a amphipathic glycoside are found widely distributed, in higher plants but are also found in some animal sources, like the marine invertebrates. Saponins exercise a wide range of pharmacological activities like expectorant, vasoprotective, antiinflammatory, hypocholesterolemic, hypoglycaemic, immunomodulatory, antifungal, antiparasitic and many others (Sparg *et al*. 2004; Sahu *et al*. 2008). For the production of steroidal hormones the pharmaceutical industry are using steroidal sapogenins as an economically important raw material since many years back (Blunden *et al*. 1975). Saponins are found to have both positive as well as negative effect on the viability of human sperm cells with *Sesbiania sesban* saponin having spermicidal at 1.0-1.3 mg/ml (Dorsaz *et al.* 1988) while ginseng saponin are found to increase progression as well as motility of sperm (Chen *et al.* 1998). The saponin extract from plants that have a negative influence on the reproduction has been long known and has been ascribed to their antizygotic, abortifacient and anti-implantation properties (Stolzenberg *et al.* 1976).

5 Conclusion

The present work summarized the proven literature about the different types of bioactive components present in the

above mentioned plants which are being traditionally used by the Mising tribes of Assam (India) for curing reproductive ailments. The curative properties of these plants may depend mainly upon these phytochemical which are described above. These herbal medicinal plants have different pharmacological activities in different animal models. Further research using recent techniques and instrumentation is required to isolate the active compounds present in these plants and to make preparation of these herbal plants in scientific manner so that their pharmacological effect can be ascertained.

Conflict of Interest

Author declares that there is no conflict of interest.

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